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Final Progress Report

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by

R. S. Manly

A Study of the Influence of ~~Normal~~ Normal Variations in Composition of External Solutions on the Equilibrium pH of Synthetic Dental Plaques

Tufts College Dental School

136 Harrison Avenue

Boston 11, Massachusetts

Summary

Investigations during the period of January 1, 1953 to June 30, 1953 were chiefly concerned with the rate of acid production by salivary sediment as effected by variations in the type of substrate and the method of application of the substrate to sediment. Details are given in the semi-annual progress report for that period. The general findings were that moderate glucose concentrations ranging from 0.6 to 6 per cent caused the same pH drop in salivary sediment. For concentrations lower than 0.6 per cent the pH differentials were proportional to concentration. The disaccharides, sucrose, maltose, and lactose produced acid equivalent to twice the concentration of monosaccharides, probably indicating that the hydrolysis of the disaccharides takes place more rapidly than does glycolysis. The hexoses, glucose, mannose, levulose and galactose were converted to acid at similar rates but there was suggestive evidence that galactose is slightly less easily hydrolyzed. The less common sugars arabinose, sorbose, rhamnose and xylose could not be used for acid production.

The effect of contact with glucose solution was persistent. The pH of salivary sediment was depressed by a 10 minute contact by glucose solution and returned to the original pH slowly in proportion to logarithm of time after contact. The initial pH did not differ much after contact with 0.2, .02, and 0.002 molar glucose solutions, but the higher concentration kept the pH depressed for a longer period of time. The concentration of sugar had more effect on the duration of the pH drop than on the magnitude of the pH drop.

Since the last report the attached paper has been prepared for publication. Additional studies have been made on the influence of temperature, time and concentration of glucose contact on the pH drop of

salivary sediment, and on the relation between thickness and the effect of inhibitors, buffer and agitation.

General and Specific Objectives of the Problem

In January 1953 we knew how to make a type of artificial dental plaque from saliva and how to use special equipment available at that time for study of acid production. The general objective of the investigation has been to study the fundamental factors that will influence acid production in an artificial dental plaque. The factors have included agitation, the thickness of the layer of salivary sediment, the concentration of different buffer solutions, the amount and kind of substrate, and the concentration frequency and duration of contact with sugar. Our general goal has been to understand quantitatively how these factors affect the pH of a plaque-like deposit containing oral microorganisms.

Details of Findings

The equipment used in these studies was described in the previous progress report and in a paper which has appeared in the August issue of the Journal of Dental Research.

In the previous report, details were given concerning tests of the effect of different concentrations of glucose for a short contact time. The striking observation was the similarity of the effects of the concentrations studied: Even though sugar concentration varied one hundred fold, the time for recovery of pH effect to the normal level was merely four fold. The concentration of sugar was found to have more effect on the duration of pH drop than upon its magnitude. These studies have been amplified by tests on 4 per cent sugar with times of contact consisting of 2, 6, and 18 minutes. In general, the longer the time of contact with glucose, the greater was the delay in recovery, but it was clear that the acidity of a synthetic plaque

would be kept low for a longer period of time by 9 two minute contacts spaced one-half hour apart, than it would be by a single 18 minute contact.

The question of mathematical relationship between buffer capacity, sediment thickness, and plaque drop has been re-evaluated during the last two months and a new technique developed for measuring the thickness of a salivary sediment in position on a glass electrode.

A series of tests were run recently in hopes of being able to evaluate simultaneously the way in which sediment thickness affects the pH differential and resistance to inhibitors, and the interaction between thickness and buffer concentration. The thickness was measured by clamping electrodes onto a mechanical stage of a microscope and setting the end of electrode in line with the cross hair in the ocular. The position of the end of the electrode was read to the nearest tenth millimeter on the micrometer stage. After a thimble of sediment had been placed on the electrode, the electrode was again clamped into the same position on the mechanical stage and the difference between readings indicated the thickness. Different thicknesses were obtained by using different volumes of salivary sediment in the thimble ranging from 0.01 to 0.16 milliliters. Sometimes there would be a good correspondence between the observed thickness and the volume used and other times there was not. After the initial pH differential was attained, a stirrer was started in the solution, then stopped, the buffer capacity of the solution was changed and restored, and finally 0.001 per cent iodoacetate was added to check the susceptibility to the inhibitor. The general findings were that the pH differential was proportional to some function of the thickness, that agitation raised the pH generally by about 0.2 units, and that the inhibitor was much less active on a thick sediment than on a thin sediment. Unfortunately the precision of the test was not

sufficient to permit this complicated procedure to be efficient. Hence it was necessary to simplify the experiment plan and to evaluate the influence of each factor separately, using sediments with different thickness.

The effect of temperature was evaluated on sediments ranging in thickness from .4 millimeters to 1.3 millimeters and tested during 1 day's time over a range of temperatures from 9° to 50°C. The lowest sediment pH occurred at 35 to 40° and rose linearly with lower temperature to 9°C. The slopes of all curves were about the same, indicating that a 10° drop in temperature would raise the pH of a sediment by approximately 0.3 pH units. At 50° the sediments were partially inactivated, with greater change taking place in the thick sediments which had the lowest pH at the time of treatment. Very similar findings were obtained on a duplicate run over the range of 11 to 55°C, except that 55°C caused considerably greater inactivation than 50°C. Again the lowest pH was attained between 30 and 40°C, and pH rose as temperature fell. The study of temperature changes support our hypothesis that the pH measurements are related to the enzymatic activities of organisms in the salivary sediment.

The interrelation between buffer capacity, thickness and pH differential was evaluated over the range of buffer capacity from 0.003 to 0.090 molar phosphate buffer. A generalized empirical curve found to fit the data = pH of the sediment equal 7.60 plus 1.2T (0.1 + log B). T is the thickness in mm and B the phosphate molarity. This fits the observed values better than any theoretical equation involving diffusion of ions which has been tested to date. Additional studies will be made in order to be certain that the constants for the equation are proper. A search of literature and study will be necessary in order to find whether there is theoretical basis for this equation or whether there is a theoretical equation

which will satisfy the experimental observations.

This equation points out and summarized in quantitative form what has been reported by this investigator previously. The data from which the equation was obtained is given on the attached figure 1. If dental plaque behaves like salivary sediment, the pH drop it will produce will be profoundly influenced by the thickness of the plaque, and by the buffer capacity of the saliva. Since the logarithm of B is generally negative, a plaque one millimeter thick would be expected to have 10 times the drop in pH as a plaque one-tenth of a millimeter thick. The influence of thickness can be overcome by a large change in buffer capacity. When sediments are thick the differential is directly proportional to buffer capacity and is considerably affected by it. When the sediments are thin the thickness sets a definite limit on pH drop until the buffer capacity goes to 0.01 molar. Beyond this thin sediment increase in differential just as rapidly as thick sediments. This study has been directed toward the goal of being able to predict from a known pH drop at one thickness and one buffer strength, the pH of the sediment at any other value of thickness or buffer capacity.

Figure 1

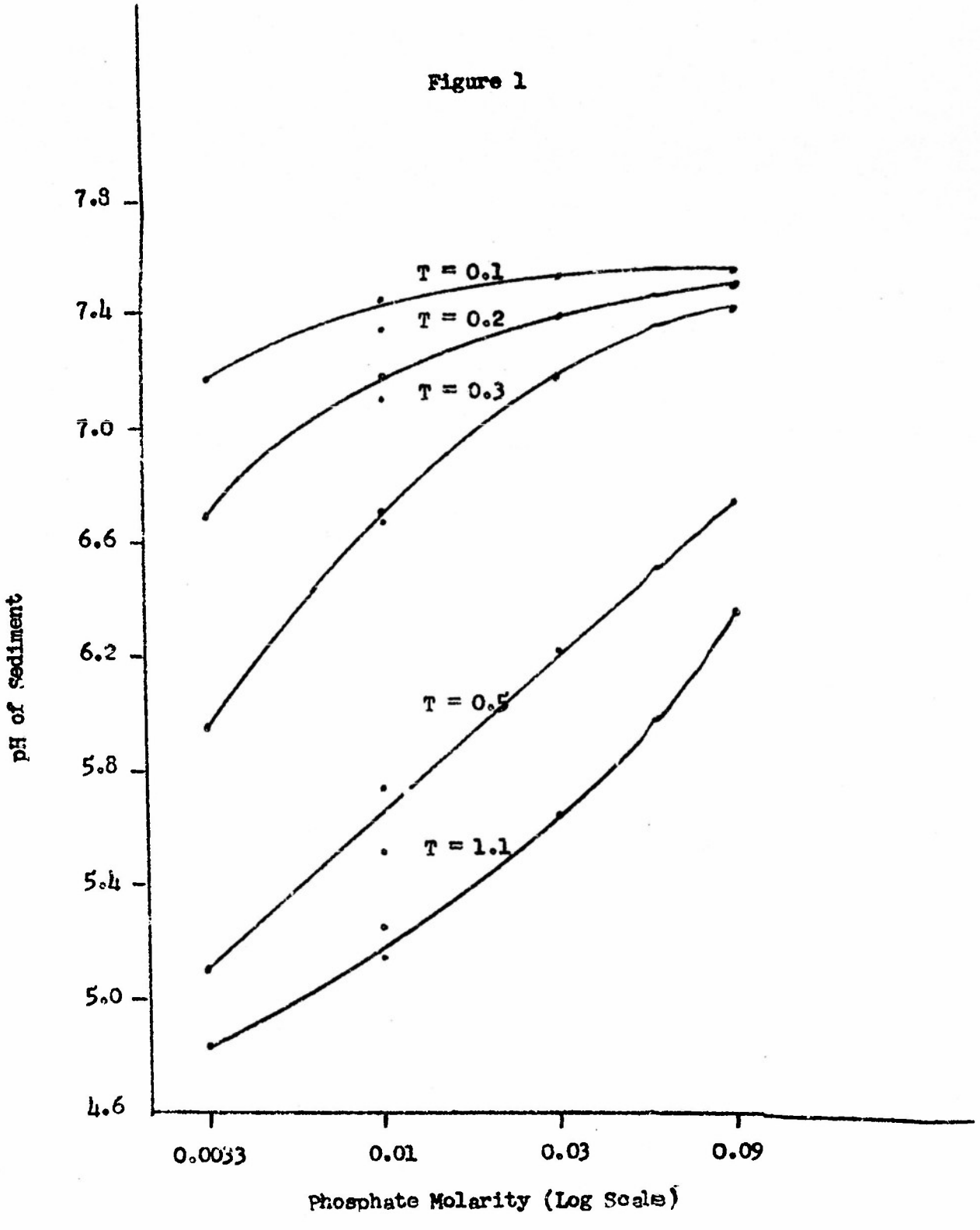
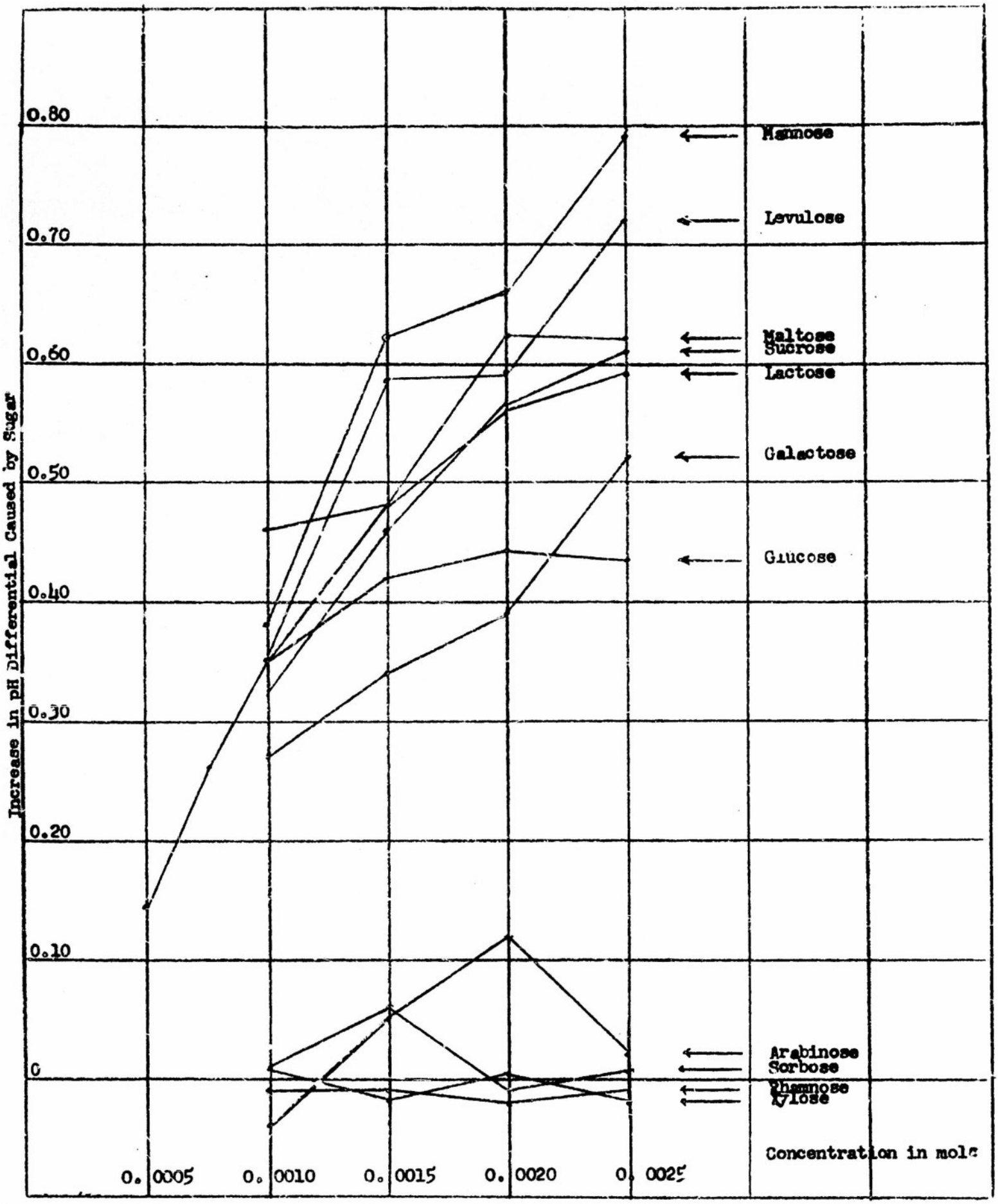

7.8
7.4
7.0
6.6
6.2
5.8
5.4
5.0
4.6
pH of Sediment
T = 0.1
T = 0.2
T = 0.3
T = 0.5
T = 1.1
0.0033
0.01
0.03
0.09
Phosphate Molarity (Log Scale)

Figure 2


0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
Increase in pH Differential Caused by Sugar
Mannose
Levulose
Maltose
Sucrose
Lactose
Galactose
Glucose
Arabinose
Sorbose
Rhamnose
Xylose
0.0005
0.0010
0.0015
0.0020
0.0025
Concentration in mole